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MicroPatent Report

PRODUCTION OF L-PHENYLALANINE BY FERMENTATION

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MATSUI YUTAKA**[21] Application No.:** JP03307614**[22] Filed:** 19911122**[No drawing]****[43] Published:** 19930330**[30] Priority:** JP 02337221 19901130**Go to Fulltext****[57] Abstract:**

PURPOSE: To produce L-phenylalanine by a fermentation process in high efficiency by culturing a microorganism transformed with a specific recombinant DNA and collecting the L-phenylalanine produced in the medium. CONSTITUTION: A microorganism is transformed with a recombinant DNA containing a gene coding an enzyme free from feed-back inhibition activity and obtained by substituting an amino acid residue in prephenate dehydratase with other amino acid residue or depleting the amino acid residue. The transformed microorganism is cultured in a medium and the produced L-phenylalanine is separated from the medium. COPYRIGHT: (C)1993,JPO&Japio

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(54) Recombinant DNA sequences encoding feedback inhibition released enzymes, plasmids comprising the recombinant DNA sequences, transformed microorganisms useful in the production of aromatic amino acids, and a process for preparing aromatic amino acids by fermentation.

(57) A method for releasing feedback inhibition of the key enzymes in the production of aromatic amino acids by fermentation is disclosed. Aromatic amino acids are prepared by a process which comprises transforming a microorganism with a recombinant DNA sequence bearing a gene or gene group encoding a feedback inhibition-released enzyme in the phenylalanine and/or tryptophan biosynthetic pathway, obtained by substituting one or two amino acid residue(s) or deleting one or more amino acid residue(s) of 3-deoxy-D-arabinoheptulonic acid 7-phosphate synthase (DS) or prephenate dehydratase, culturing the microorganism and isolating the aromatic amino acid produced in the medium. Higher efficiency and improved yields in the production of L-phenylalanine and L-tryptophan by fermentation are realized.

EP 0 488 424 A2

BACKGROUND OF THE INVENTIONField of the Invention:

5 The present invention relates to recombinant DNA sequences encoding feedback inhibition released enzymes, plasmids containing these recombinant DNA sequences, microorganisms transformed with these plasmids, and a process for preparing L-tryptophan, L-phenylalanine and L-tyrosine by fermentation.

Discussion of the Background:

10 Demand for aromatic amino acids is rapidly increasing. For example, L-phenylalanine is used as a raw material for the sweetener aspartame, L-tryptophan is an important feed additive, and all three (L-phenylalanine, L-tryptophan, and L-tyrosine) are useful as transfusion drugs.

15 Many methods for preparing aromatic amino acids using microorganisms are known. For example, methods for preparing L-phenylalanine using recombinant Escherichia coli are described in Japanese Published Unexamined Patent Application Nos. 56-1890, 58-103398, 61-92565 and 1-104160, and World Patent Publication WO 87/00202. A method for preparing L-phenylalanine or L-tyrosine using a mutant belonging to Coryneform bacteria is described in Japanese Patent Published Unexamined Application No. 61-128897, and methods using recombinant Coryneform bacteria are described in Japanese Unexamined 20 Published Patent Application Nos. 60-34197, 60-24192, 61-260892 and 61-124375. A method for preparing L-tryptophan using recombinant E. coli is described in Japanese Published Unexamined Patent Application No. 57-71397 and U.S. Patent No. 4,371,614; methods using mutants of Bacillus subtilis are described in Japanese Published Unexamined Patent Application Nos. 53-39517 and 62-34399; methods using recombinant Bacillus subtilis are described in Japanese Patent Published Unexamined Application Nos. 61-25 104790 and 62-34399; methods using a mutant of Coryneform bacteria are described in Japanese Published Unexamined Patent Application No. 57-174096; and a method using recombinant Coryneform bacteria is described in Japanese Published Unexamined Patent Application No. 62-51980.

30 Generally, in the biosynthetic route of aromatic amino acids, a key enzyme which plays a central role in the biosynthesis is subject to feedback inhibition by the final product. In the methods described above, the desired amino acids are principally produced using microorganisms wherein the key enzyme is released from feedback inhibition by the final product. The key enzymes released from feedback inhibition in the above methods include 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase (hereafter abbreviated as "DS") and prephenate dehydratase (hereafter abbreviated as "PD").

35 Turning first to DS, among the microorganisms used in the methods described above, Escherichia coli has three types of naturally-occurring (wild-type) DS isozymes. These isozymes are encoded by genes called aroF, aroG and aroH, which are subject to feedback inhibition by L-tyrosine, L-phenylalanine and L-tryptophan, respectively.

40 The nucleotide sequences and amino acid sequences relevant to these genes and enzymes are already reported [aroF: Hudson, G.S. and Davidson, B.E., *J. Mol. Biol.*, 180, 1023 (1984); aroG: Davies, W.D. and Davidson, B.E., *Nucleic Acids Res.*, 13, 4045 (1982); aroH: Ray, J.M. et al., *J. Bacteriol.*, 170, 5500 (1988)].

45 In order to efficiently produce the desired aromatic amino acids, expression of these DS genes must be improved. With respect to aroH-encoded DS, feedback inhibition by L-tryptophan has been released using mutant aroH [Ray, J.M. et al., *J. Bacteriol.*, 170, 5500 (1988)]. However, the DS activity derived from aroH is very poor, and the aroH-derived DS is unsuitable for improvement by recombinant DNA techniques. It is more efficient to utilize aroF- or aroG-encoded DS in which feedback inhibition is released ("feedback inhibition-released" DS).

50 An example of a mutation which releases feedback inhibition of aroF-encoded DS by L-tyrosine is the substitution of the 148 proline residue from the N-terminus (¹⁴⁸Pro) with a leucine residue [Weaver, L.M. and Herrmann, K.M., *J. Bacteriol.*, 172, 6581 (1980)].

55 Only a few examples as shown below for the production of aromatic amino acids by fermentation employ feedback inhibition-released DS with a clearly shown mutation site. Edwards et al. teach that feedback inhibition by L-tyrosine in the production of L-phenylalanine by fermentation is released by substituting the 152 glutamine residue (¹⁵²Gln) of DS encoded by aroF with isoleucine [WO 87/00202]. Furthermore, Sinenki et al. teach that feedback inhibition by L-phenylalanine in the production of L-phenylalanine by fermentation is suppressed by substituting the 76 leucine residue (⁷⁶Leu) of DS encoded by aroG with valine [Japanese Published Unexamined Patent Application No. 58-103398]. However, the enzyme activity of the feedback inhibition-released DS and the amount of L-phenylalanine produced are unknown. No reports of the production of L-tryptophan by feedback inhibition-released DS mutants are

known.

Turning next to PD, a wild-type bifunctional enzyme (CM-PD) present in Escherichia coli having both chorismate mutase (hereafter abbreviated as "CM") activity and PD activity is subject to feedback inhibition by L-phenylalanine. The enzyme is encoded by a gene called pheA. The nucleotide sequence of pheA and the amino acid sequence of wild-type CM-PD are known [Hudson, G.S. and Davidson, B.E., *J. Mol. Biol.*, 180, 1023 (1984)]. In order to efficiently produce L-phenylalanine, it is important to release the feedback inhibition of CM-PD by L-phenylalanine.

Some examples of modification and mutation on an amino acid level are known to release feedback inhibition for the fermentative production of L-phenylalanine. By modifying two tryptophane residues (226 and 338 amino acids from the N-terminus) of CM-PD with dimethyl(2-hydroxy-5-nitrobenzylsulfonium bromide), an enzyme having resistance to feedback inhibition can be obtained [Gething, M.J.H. and Davidson, B.E., *Eur. J. Biochem.*, 78, 111 (1977)]. Feedback inhibition-released enzyme can be obtained by deleting the 338 tryptophan residue (³³⁸Trp) or substituting ³³⁸Trp and the subsequent residues with arginine-glycine (Japanese Published Unexamined Patent Application No. 1-235597). Inserting the amino acid sequence tryptophan-arginine-serine-proline into the site of the same 338 tryptophan residue also releases feedback inhibition (WO 87/00202). These techniques focus on the 338 tryptophan residue. However, no definitive study on the effects of modifying or mutating the ²²⁶Trp residue has been performed.

On the other hand, in Coryneform bacteria, PD is subject to feedback inhibition by L-phenylalanine. A gene in which the feedback inhibition by L-phenylalanine has been released is known. [Ozaki, A. et al., *Agric. Biol. Chem.*, 49, 2925 (1986); Ito, H. et al., *Appl. Microbiol. Biotechnol.*, 33, 190 (1989)]. The nucleotide sequence of the wild type Coryneform PD gene shows homology to the pheA gene of Escherichia coli K-12 [Follettie, M.T. and Sinsky, A.J., *J. Bacteriol.*, 167, 695 (1986)]. However, the nucleotide sequence of the feedback inhibition-released PD gene in Coryneform bacteria is unknown, as is mutation of the nucleotide sequence and release of feedback inhibition by substitution of the corresponding amino acid sequence.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a process for efficiently preparing an aromatic amino acids by fermentation.

A further object is to provide transformed microorganisms useful in the production of aromatic amino acids by fermentation.

A further object is to provide recombinant plasmids which express genes encoding key enzymes in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

A further object is to provide recombinant DNA sequences which encode key enzymes in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

A further object is to provide novel recombinant enzymes which are important in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

These and other objects which will become apparent during the following detailed description of the preferred embodiments have been accomplished by a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released, a plasmid comprising the recombinant DNA sequence, a microorganism useful in the production of aromatic amino acids transformed with one or more of the plasmids, and a process for preparing an aromatic amino acid which comprises culturing the transformed microorganism and isolating the aromatic amino acid produced thereby.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Fig. 1 shows the construction of the plasmids pTS-aroF and pTS-aroG;

Fig. 2 shows the extent of inhibition by L-tyrosine of activity in DS encoded by both wild-type and mutant aroF;

Fig. 3 shows the extent of inhibition by L-phenylalanine of activity in DS encoded by both wild type and mutant aroG.

Fig. 4 shows the extent of inhibition by L-phenylalanine in the prephenate dehydratase activity of both wild-type and mutant chorismate mutase-prephenate dehydratase;

Fig. 5 shows the construction of the plasmid pACKG4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 The present invention relates to a mutant feedback inhibition-released enzyme in the biosynthetic pathway of aromatic amino acids; a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; a plasmid comprising a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; a microorganism useful in the production of aromatic amino acids transformed with
 10 one or more plasmids comprising a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; and a process for preparing an aromatic amino acid which comprises culturing a microorganism transformed with one or more plasmids comprising a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released, and isolating the aromatic amino acid produced thereby.
 15 In the present application, the phrase "aromatic amino acid" refers to L-phenylalanine, L-tryptophan and L-tyrosine. Also, an enzyme is "released" from feedback inhibition by a final product if the activity of the enzyme doesn't decrease in the presence of the final product.

20 Preferably, the enzymes of the present invention in the biosynthetic pathway of aromatic amino acids which are to be released from feedback inhibition are 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase (DS), prephenate dehydratase (PD) and chorismate mutase-prephenate dehydratase (CM-PD). The means by which each of these enzymes is released from feedback inhibition in the present invention is preferably by mutation, wherein one or two amino acid residue(s) are substituted with other amino acid residue(s) or one or more amino acid residue(s) are deleted. Furthermore, the transformed microorganism preferably belongs to the genus *Escherichia*, and is preferably transformed with one or more plasmids bearing a recombinant DNA sequence corresponding to one of the above mutant enzymes.

25 Firstly, the present Inventors have acquired a novel gene encoding DS, wherein the feedback inhibition is released by cloning the natural DS gene of *Escherichia coli* and subjecting the cloned gene to mutation. Furthermore, the natural PD gene of *Brevibacterium lactofermentum* is cloned, and a gene encoding PD in which feedback inhibition is released, is cloned from L-phenylalanine-producing *Coryneform* bacteria. Even
 30 further, the natural CM-PD gene of *Escherichia coli* is cloned, and the cloned gene is then subjected to mutation to produce a novel gene encoding CM-PD in which feedback inhibition is released. By transfecting or transforming phenylalanine-producing bacteria with one or two of the genes of the present invention, production of L-phenylalanine by fermentation can be improved.

35 The present Inventors have also improved the fermentative production of L-tryptophan by transforming a microorganism with the novel DS gene of the present invention in combination with a tryptophan operon, in which the feedback inhibition of anthranilate synthase (hereafter abbreviated as AS), an enzyme for the L-tryptophan biosynthesis system, is also released.

The novel gene of the present invention encoding DS having released feedback inhibition is prepared by the following procedure.

40 Firstly, *aroF* and *aroG* genes are cloned from chromosomal DNA of *Escherichia coli* using the PCR method as described in U.S. Patent Nos. 4,800,159, 4,683,202 and 4,683,195, all incorporated herein by reference. The chromosomal DNA appropriate as a source of the *aroF* and *aroG* genes for use in the present invention may be cloned from any strain of *Escherichia coli*, but the preferred strain is K-12 MC1061 (ATCC 53338). The desired genes are then mutated with hydroxylamine by a known method; for
 45 example, that described in J. Mol. Biol., 175, 331 (1984).

The genes *aroF* and *aroG* encode DS which is subject to feedback inhibition by L-tyrosine and L-phenylalanine, respectively, and also include mutants caused by genetic polymorphism, etc. Genetic polymorphism refers to a modification of an amino acid sequence of a protein due to natural mutation of a gene.

50 In order to cause mutation of the gene, a number of effective methods are known. Examples include recombinant PCR methods [PCR Technology, Stockton Press (1989)], site specific mutation [Kramer, W. and Frits, H.J., Methods in Enzymology, 154, 350 (1987)], conventional methods exposing a strain bearing the gene to UV (ultraviolet light) rays, conventional methods treating the DNA or DNA-bearing microorganism with a chemical (N-methyl-N'-nitrosoguanidine, nitric acid, etc.), and conventional methods for chemical synthesis of the desired gene, such as those employing a known automated synthesizer.

In the present invention, the mutated amino acid residue of DS is in the region of the amino acid sequence which participates in the mechanism of feedback inhibition by L-tyrosine, L-phenylalanine or L-tryptophan. For example, in DS encoded by *aroF*, the 147 aspartic acid residue (¹⁴⁷Asp) and the 181 serine

residue (¹⁸¹Ser) from the N-terminus are the mutated amino acid residues. Any mutation of the amino acid residue which results in release from feedback inhibition is suitable. For example, substitution, deletion, or addition is suitable. The DS mutations and the corresponding nucleotide sequence mutations exemplified in the present invention are summarized in Table 1.

5 By transfecting a suitable microorganism with the mutant aroF or aroG gene above as a recombinant DNA sequence, the microorganism can express the recombinant mutant gene in which feedback inhibition is released.

In the present invention, the novel gene encoding PD in Brevibacterium lactofermentum and the novel gene encoding CM-PD in Escherichia coli were prepared as follows.

10 Firstly, the nucleotide sequence of the Brevibacterium lactofermentum PD gene encoding PD in which feedback inhibition by L-phenylalanine is released was determined and analyzed. It has thus been found that the L-phenylalanine-producing strain expressed PD in which one amino acid is substituted, as compared to the wild strain. Next, based on this finding, a substitution or a deletion of amino acid residue(s) was carried out at the corresponding position of CM-PD in Escherichia coli K-12, resulting in CM-PD in which the feedback inhibition is released.

15 Enzymes having PD activity referred to in the present invention refer to enzymes derived from microorganisms such as Coryneform bacteria having PD activity, and further refer to enzymes derived from microorganisms such as Escherichia coli, etc., having the bifunctional activity of CM-PD.

In the present invention, the mutated amino acid residue of PD refers to a substitution of an amino acid residue or a deletion of amino acid residue(s) present in the region of the amino acid sequence which participates in the mechanism of feedback inhibition by L-phenylalanine. For example, in PD derived from Brevibacterium lactofermentum, the 235 serine residue (²³⁵Ser) is suitable for mutation, and in CM-PD derived from Escherichia coli, the 330 serine residue (³³⁰Ser) from the N-terminus is an amino acid residue suitable for mutation. Suitable mutations include any which result in the release of feedback inhibition, but particularly suitable mutations include substitutions of ²³⁵Ser or ³³⁰Ser with proline or aspartic acid residue, or deletion of amino acid residues down stream from ³³⁰Ser.

By transfecting a suitable microorganism with the mutant PD or CM-PD gene described above as a recombinant DNA sequence, expression of PD in which feedback inhibition is released is achieved in the transfected microorganism.

20 The recombinant DNA sequences obtained by the foregoing procedures refer to those obtained by incorporating a gene encoding feedback inhibition-released DS or PD into a vector of plasmid or phage DNA. In the present invention, promoters such as lac, trp, PL, etc. which act in the microorganism may also be used to efficiently perform the expression of the gene. The recombinant DNA sequences referred to herein include those obtained by incorporating one or more of the above-described genes into a chromosome according to known methods. Examples include methods using a transposon (Berg, D.E. and Berg, C.M., Bio/Technol., 1, 417 (1983)), Mu phage (Japanese Published Unexamined Patent Application No. 2-109985) or homologous recombination [Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972)].

25 As the microorganism containing the recombinant DNA, any microorganism may be used, irrespective of species and strain of the microorganism, so long as it expresses the gene encoding the desired enzyme (such as DS or PD) and is capable of producing the aromatic amino acid (for example, in the case of L-phenylalanine, the microorganism which has acquired L-phenylalanine productivity by imparting L-phenylalanine analog resistance thereto). Particularly suitable microorganisms are selected from the genus Escherichia, the genus Brevibacterium, the genus Corynebacterium, the genus Bacillus, the genus Serratia, the genus Pseudomonas, etc.

30 The thus obtained microorganism transformed by the recombinant DNA bearing the feedback inhibition-released DS or PD gene is cultured, the desired aromatic amino acid is produced by the transformed microorganism in a suitable medium, and the accumulated aromatic amino acid is collected and isolated.

The medium used for producing the aromatic amino acid is a conventional medium containing appropriate carbon sources, nitrogen sources, inorganic ions and, if necessary, other organic components.

35 Suitable carbon sources include sugars such as glucose, lactose, galactose, fructose, starch hydrolysate, etc.; alcohols such as glycerol, sorbitol, etc.; organic acids such as fumaric acid, citric acid, succinic acid, etc.

40 Suitable nitrogen sources include inorganic ammonium salts such as ammonium sulfate, ammonium chloride, ammonium phosphate, etc.; organic nitrogen such as soybean hydrolysate, etc.; ammonia gas, ammonia water, etc.

45 Suitable organic trace nutrient sources preferably are present, and include required substances such as vitamin B₁, L-tyrosine, or yeast extract, etc., in an appropriate amount.

In addition thereto, small amounts of potassium phosphate, magnesium sulfate, iron ions, manganese ions, etc. may be present.

Incubation is carried out for 16 to 72 hours under aerobic conditions. The temperature for incubation is maintained between 30 and 45°C and the pH is maintained in the range of 5 to 7 during the incubation.

5 The pH may be adjusted with either acids or alkaline substances, which may be inorganic or organic, or may be adjusted with ammonia gas, etc., as is appropriate to maintain the desired pH and concentrations of components in the medium.

10 The desired aromatic amino acid is isolated from the fermentation medium generally by conventional methods, such as use of an appropriate ion exchange resin, precipitation, and/or other known techniques, either alone or in combination.

15 By the general process described above, the transformant expressing feedback inhibition-released DS, PD and/or CM-PD is obtained, and by culturing the transformant, the productivity of aromatic amino acids can be greatly improved.

20 Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention, and are not intended to be limiting thereof.

Example 1: Preparation of a novel gene encoding DS in which the feedback inhibition is released

25 (1) Collection of an aroF-derived mutant DS gene of Escherichia coli

Chromosomal DNA was extracted from Escherichia coli K-12 MC1061 strain in a conventional manner. In a separate procedure, two synthetic DNA primers shown by Sequence Nos. 1 (SEQ ID NO:1) and 2 (SEQ ID NO:2) were synthesized in a conventional manner, based on the known nucleotide sequence of the target 25 aroF gene [J. Mol. Biol., 180, 1023 (1984)].

30 **Sequence No. 1 GCTAACCAAGT AAAGCCAACA (SEQ ID NO:1)**

Sequence No. 2 CCCACTTCAG CAACCAGTTC (SEQ ID NO:2)

35 These primers have homologous sequences upstream and downstream from the aroF gene. Using the chromosomal DNA and the DNA primers, PCR (polymerase chain reaction) is conducted according to the method of Erlich et al. [PCR Technology, Stockton Press (1989)], yielding a DNA fragment of 1.5 Kbp. Thereafter, as shown in Fig. 1, left side, the fragment is cleaved with restriction enzymes EcoRV and Eco47III, and the product is then ligated with the SmaI digestion product of pHSG398 (manufactured by 40 Takara Shuzo) using T4 DNA ligase. Competent cells of Escherichia coli JM109 strain (manufactured by Takara Shuzo) were transformed with the reaction mixture. A plasmid having the aroF gene was extracted from the strains resistant to chloramphenicol to yield the plasmid pHSG-aroF.

45 Subsequently, pHSG-aroF was digested with restriction enzymes EcoRI and HindIII, and the resulting DNA fragment bearing the aroF gene was ligated with the EcoRI and HindIII digestion fragment of plasmid pTS1 (Japanese Patent Application No. 2-192162) using T4 DNA ligase. Competent cells of DS-deleted (aroF, aroG, aroH) strain AB3257 of Escherichia coli K-12 were transformed with the reaction mixture. The AB3257 strain was acquired from the Escherichia coli Genetic Stock Center. From among the strains 50 resistant to ampicillin, the strain in which auxotrophy of L-tyrosine, L-phenylalanine and L-tryptophan disappeared was selected, and a plasmid was extracted therefrom, yielding plasmid pTS-aroF.

Next, after mutation of plasmid pTS-aroF using hydroxylamine according to the method of J. Mol. Biol., 175, 331 (1984), the mutant was used to transform the E. coli AB3257 strain. After ampicillin-resistant 55 strains were collected, two strains which grew in minimum medium supplemented with 1 mM L-tyrosine were selected. From these strains, plasmids pTS-aroF15 and pTS-aroF33 bearing the genes encoding feedback inhibition-released DS were obtained.

Cells of AB3257 strain transformed with plasmids containing the gene encoding non-feedback inhibition-released DS are subject to feedback inhibition at 1 mM concentration of L-tyrosine in the minimum medium. Accordingly, the strain subject to feedback inhibition failed to synthesize aromatic amino acids such as L-phenylalanine or L-tryptophan, and failed to grow.

(2) Preparation of aroG-derived mutant DS gene of Escherichia coli

A mutant aroG gene was collected in a manner similar to the case of the aroF gene. Two synthetic DNA primers shown by Sequence Nos. 3 (SEQ ID NO:3) and 4 (SEQ ID NO:4) were synthesized in a conventional manner, based on the known nucleotide sequence of the aroG gene (Nucleic Acids Res., 10, 4045 (1982)).

5

Sequence No. 3 **GTATTTACCC CGTTATTGTC (SEQ ID NO:3)**

10

Sequence No. 4 **ACTCCGCCGG AAGTGACTAA (SEQ ID NO:4)**

15

Using the primers and the chromosomal DNA of the E. coli MC1061 strain, PCR was carried out to obtain a DNA fragment of 2.1 Kbp. As shown in Fig. 1, right side, the fragment was cleaved with restriction enzymes Sall and Eco47III, and the product was then ligated with the Sall and SmaI digestion product of pHSG398 (manufactured by Takara Shuzo) using T4 DNA ligase. Competent cells of Escherichia coli JM109 strain were transformed with the reaction mixture. From among the strains resistant to chloramphenicol, a plasmid having the aroG gene was extracted to yield the plasmid pHSG-aroG.

20

Subsequently, pHSG-aroG was digested with restriction enzymes EcoRI and HindIII, and the resulting DNA fragment bearing the aroG gene was ligated with the EcoRI and HindIII digestion fragment of plasmid pTS1 using T4 DNA ligase. From among the grown strains resistant to ampicillin, the strain in which auxotrophy of L-tyrosine, L-phenylalanine and L-tryptophan disappeared was selected, and a plasmid was extracted therefrom to yield the plasmid pTS-aroG.

25

Next, after mutation of the plasmid using the hydroxylamine method of Example 1-(1) above, the mutant plasmid was used to transform competent cells of the E. coli AB3257 strain. After ampicillin-resistant strains were isolated, 6 strains which grew in minimum medium supplemented with 10 mM L-phenylalanine were selected. From these strains, plasmids pTS-aroG4, pTS-aroG8, pTS-aroG15, pTS-aroG17, pTS-aroG29 and pTS-aroG40 bearing the aroG gene encoding feedback inhibition-released DS were obtained.

30

In cells of the AB3257 strain expressing the non-feedback inhibition-released DS, feedback inhibition occurs at a concentration of 10 mM L-phenylalanine in minimum medium. Accordingly, the non-feedback inhibition-suppressed strain fails to synthesize aromatic amino acids such as L-tryptophan and/or L-tyrosine, and therefore, fails to grow.

(3) Determination of DS enzyme activity

35

The above plasmids, bearing either mutant aroF (pTS-aroF15 and pTS-aroF33) or mutant aroG (pTS-aroG4, pTS-aroG8, pTS-aroG15, pTS-aroG17, pTS-aroG29 and pTS-aroG40), were used to transform Escherichia coli AB3257 strain having no DS activity. The respective transformants were named AJ 12598 (AB3257/pTS-aroF15), AJ 12599 (AB3257/pTS-aroF33), AJ 12562 (AB3257/pTS-aroG4), AJ 12600 (AB3257/pTS-aroG8), AJ 12563 (AB3257/pTS-aroG15), AJ 12601 (AB3257/pTS-aroG17), AJ 12602 (AB3257/pTS-aroG29) and AJ 12603 (AB3257/pTS-aroG40), respectively. Among them, AJ 12563 and AJ 12603 were deposited as representative strains in the Fermentation Research Institute of the Agency of Industrial Science & Technology of Japan, under the deposit numbers Escherichia coli FERM BP-3567 and FERM BP-3568, respectively. For the purpose of comparison, plasmids bearing wild type genes were also used to transform the E. coli AB3257 strain.

45

Each of these strains were cultured for 24 hours in a known L-phenylalanine-producing medium [Sugimoto, S. et al., J. Biotechnol., 5, 237 (1988)]. From the culture cells, the crude enzyme solution was prepared by ultrasonic homogenization. The enzyme activity of DS was determined in a conventional manner [Gollub, E. et al., Methods Enzymol., 17, 349], in the presence of L-tyrosine in the case of aroF, and in the presence of L-phenylalanine in the case of aroG. The results presented in Figs. 2 and 3 show that the DS enzyme activity of the wild type transformants (Escherichia coli AB3257/pTS-aroF) is strongly inhibited in the presence of L-tyrosine, whereas the respective mutant transformants are released from feedback inhibition by L-tyrosine. Likewise, in the wild type transformant Escherichia coli AB3257/pTS-aroG, the enzyme activity is strongly inhibited in the presence of L-phenylalanine, whereas in the respective mutant transformants, feedback inhibition by L-phenylalanine is released. Furthermore, the mutant strain AJ 12562 not only releases feedback inhibition by L-phenylalanine, but surprisingly, the DS enzyme activity increases as the concentration of L-phenylalanine increases.

(4) Determination of the mutation site of DS in which the feedback inhibition is released

The nucleotide sequences of the feedback inhibition-released aroF15, aroF33, aroG4, aroG8, aroG15, aroG17, aroG29 and aroG40 were determined in a conventional manner [Molecular Cloning (Second Edition), Cold spring Harbor Press (1989)]. The specific substitution site on the amino acid sequence and the mutation site on the corresponding nucleotide sequence are shown in Table 1.

5 These sequences are all novel.

Table 1

10	Mutant Gene	Substitution Site of Amino Acid		Corresponding Nucleotide Sequence Change
		Position from N-Terminus	Amino Acid Sequence Change	
15	aroF15	147	Asp → Asn	GAT → AAT
	aroF33	181	Ser → Phe	TCC → TTC
20	aroG4	150	Pro → Leu	CCA → CTA
	aroG8	202	Ala → Thr	GCC → ACC
	aroG15	146	Asp → Asn	GAT → AAT
25	aroG17	147 332	Met → Ile Glu → Lys	ATG → ATA GAA → AAA
	aroG29	147	Met → Ile	ATG → ATA
30	aroG40	157 219	Met → Ile Ala → Thr	ATG → ATA GCG → ACG

Example 2: Preparation of a novel gene encoding PD in which the feedback inhibition is released

30 (1) Determination of the mutation site of Brevibacterium lactofermentum mutant PD

The nucleotide sequence of the Ncol fragment in plasmid pAJ16 bearing the PD gene of Brevibacterium lactofermentum wild strain was determined by the dideoxy method, using the homology to known Corynebacterium s.p. PD gene [Follettie, M.T. and Sinsky, A.J., J. Bacteriol., 167, 695 (1986)] as an index. The plasmid is harbored on Brevibacterium lactofermentum AJ 12125 (FERM P-7546). The resulting nucleotide sequence (SEQ ID NO:5) and the corresponding amino acid sequence (SEQ ID NO:6) are shown below. The B. lactofermentum PD amino acid sequence is different by only one amino acid residue from that of Corynebacterium s.p.

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Sequence 5:

5 ATGAGCGACGCACCAATTGTTGTCCTATTGGGGCTGCCGGAACCTCACCGAAGAA
MetSerAspAlaProIleValValAlaTyrLeuGlyProAlaGlyThrPheThrGluGlu
1 20

10 GCCCTCTACAAATTGCCGACGCCGGCGTATTCGGCGACGGTGAGATCGAGCAGCTACCA
AlaLeuTyrLysPheAlaAspAlaGlyValPheGlyAspGlyGluIleGluGlnLeuPro
21 40

15 GCCAAATGCCACAAGAAGCTGTCGACGCCGGTCCGCCACGGCACCGCCCAGTCGCGCGTC
AlaLysSerProGlnGluAlaValAspAlaValArgHisGlyThrAlaGlnPheAlaVal
41 60

20 GTCGCCATCGAAAACCTCGTCGACGGCCCCGTACCCCCACCTTCGACGCCCTGACCAG
ValAlaIleGluAsnPheValAspGlyProValThrProThrPheAspAlaLeuAspGln
61 80

25 GGCTCCAACGTGCAAATCATGCCGAAGAAGAACTCGATATTGCCCTTTCCATCATGGTC
GlySerAsnValGlnIleIleAlaGluGluGluLeuAspIleAlaPheSerIleMetVal
81 100

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CGGCCAGGGACTTCGCTTGCGACGTAAAACCTGCCACCCACCGGTTGGGTACCAA
 ArgProGlyThrSerLeuAlaAspValLysThrLeuAlaThrHisProValGlyTyrGln
 101
 120

5 CAAGTGAAAAACTGGATGGCAACCACCATTCCGGACGCCATGTATCTTCAGCAAGCTCC
 GlnValLysAsnTrpMetAlaThrThrIleProAspAlaMetTyrLeuSerAlaSerSer
 121
 140

10 AACGGCGCCGGCGCACAAATGGTTGCCGAAGGAACCGCCGACGCCAGCCGAGCGGCCCTCC
 AsnGlyAlaGlyAlaGlnMetValAlaGluGlyThrAlaAspAlaAlaAlaAlaProSer
 141
 160

15 CGCGCAGCCGAACCTTCGGACTGGAACGCCCTTGATGATGTCGCCGACGTCCGCCGC
 ArgAlaAlaGluLeuPheGlyLeuGluArgLeuValAspAspValAlaAspValArgGly
 161
 180

20 GCCCGCACCCGCTTCGTTGCCAGTCCAAGCCAAGCAGCCGTTCCGAACCGACCGGCCAC
 AlaArgThrArgPheValAlaValGlnAlaGlnAlaAlaValSerGluProThrGlyHis
 181
 200

25 GACCGCACCTCCGTCACTTCTCCCTACCGAATGTGCCAGGCAGCCTCGCGCGCCCTC
 AspArgThrSerValIlePheSerLeuProAsnValProGlySerLeuValArgAlaLeu
 201
 220

30 AACGAATTGCCATCCGTGGCGTCGACCTCACCCGATCGAATCCGCCCCACCGCAAA
 AsnGluPheAlaIleArgGlyValAspLeuThrArgIleGluSerArgProThrArgLys
 221
 240

35 GTCTTCGGAACCTACCGCTTCCACCTGGACATATCCGGACATATCCGCGACATCCCCGTC
 ValPheGlyThrTyrArgPheHisLeuAspIleSerGlyHisIleArgAspIleProVal
 241
 260

40 GCCGAAGCCCTCCGCGCACTCCACCTCCAAGCCGAAGAACTCGTATTGTCGGTTCTGG
 AlaGluAlaLeuArgAlaLeuHisLeuGlnAlaGluGluLeuValPheValGlySerTrp
 261
 280

45 CCCTCCAACCGTGCAGAACGACAGCACGCCAAACGACCAACTAGCTAACGTACACAAG
 ProSerAsnArgAlaGluAspSerThrProGlnThrAspGlnLeuAlaAsnValHisLys
 281
 300

50 GCGGACGAATGGGTTCGCGCAGCAAGCGAAGGAAGGAAACTTAACCTAG
 AlaAspGluTrpValArgAlaAlaSerGluGlyArgLysLeuAsn***
 301
 315

45 Next, the nucleotide sequence (SEQ ID NO: 7) of the gene on plasmid pPH14 encoding PD of the
 phenylalanine-producing strain of Brevibacterium lactofermentum was determined. The sequence shown
 below was obtained. The plasmid used was the one borne on Brevibacterium lactofermentum AJ 12259
 (FERM BP-3565). A comparison of the amino acid sequences was made between the wild-type PD and the
 feedback inhibition-released PD (SEQ ID NO: 8) and it was found that ²³⁵Ser residue of the wild strain was
 mutated to a proline residue in the feedback inhibition-released PD.

Sequence of Brevibacterium lactofermentum PD (pPH14) :

5 ATGAGCGACGCACCAATTGTTGGCCTATTGGGGCCTGCCGAAACCTCACCGAAGAA
 MetSerAspAlaProIleValValAlaTyrLeuGlyProAlaGlyThrPheThrGluGlu
 10 20
 GCCCTCTACAAATTGCCGACGCCGGCGTATTGGCGACGGTGAGATCGAGCAGCTACCA
 AlaLeuTyrLysPheAlaAspAlaGlyValPheGlyAspGlyGluIleGluGlnLeuPro
 15 21
 20 40
 GCCAAATGCCACAAGAACGCTGTCGACGCCGTCCGCCACGGCACCGCCCAGTCGCGGTG
 AlaLysSerProGlnGluAlaValAspAlaValArgHisGlyThrAlaGlnPheAlaVal
 41
 15 60
 20 45
 GTGCCATCGAAAACCTCGTCGACGGCCCCGTACCCCCCACCTCGACGCCCTGACCAG
 ValAlaIleGluAsnPheValAspGlyProValThrProThrPheAspAlaLeuAspGln
 61
 80
 20 40
 GGCTCCAACGTGCAAATCATGCCGAAGAACGAACTCGATATTGCCCTTCATCATGGTC
 GlySerAsnValGlnIleIleAlaGluGluLeuAspIleAlaPheSerIleMetVal
 81
 100
 25 45
 CGGCCAGGGACTTCGCTTGCCGACGTCAAAACCTGCCACCCACCGGTTGGGTACCAA
 ArgProGlyThrSerLeuAlaAspValLysThrLeuAlaThrHisProValGlyTyrGln
 101
 120
 30 45
 CAAGTGAAAAACTGGATGGCAACCACCATTCCGGACGCCATGTATCTTCAGCAAGCTCC
 GlnValLysAsnTrpMetAlaThrThrIleProAspAlaMetTyrLeuSerAlaSerSer
 121
 140
 35 45
 AACGGCGCCGGCGCACAAATGGTTGCCGAAGGAACCGCCGACGCCAGCCGAGCGCCCTCC
 AsnGlyAlaGlyAlaGlnMetValAlaGluGlyThrAlaAspAlaAlaAlaProSer
 141
 160
 161
 CGCGCAGCCGAACTCTCGGACTGGAACGCCPTGTTGATGATGTCGCCGACGTCCGCCGC
 ArgAlaAlaGluLeuPheGlyLeuGluArgLeuValAspAspValAlaAspValArgGly
 180
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 45
 50
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30 (2) Construction of a novel gene encoding a mutant CM-PD of *Escherichia coli*

35 Chromosomal DNA was extracted from Escherichia coli K-12 RR1 strain in a conventional manner. In a separate procedure, four synthetic DNA primers (Sequence Nos. 7-10) (SEQ ID NOS: 9-12) were chemically synthesized in a conventional manner, based on the known nucleotide sequence of the pheA gene [Hudson, G.S. and Davidson, B.E., *J. Mol. Biol.*, 180, 1023 (1984)].

40 Sequence No. 7 TCAACAAAGCT GGAACGGACG (SEQ ID NO: 9)
Sequence No. 8 CGCCCGATTAA CCGCCCTTGAG (SEQ ID NO: 10)

45 Sequence No. 9 CCGTCGGAA CCACGCCGA T (SEQ ID NO: 11)
Sequence No. 10 ATCGGGCGTG ATTCCAGACG G (SEQ ID NO: 12)

50 Sequence Nos. 7 and 8 have homologous sequences upstream and downstream from the pheA gene, respectively. Sequence Nos. 9 and 10 are complementary to each other and have almost perfect homology to the sequence around the 330 serine residue (³³⁰Ser), except that one base pair is different, i.e., T (thymine base) is substituted with C (cytosine base). CM-PD of Escherichia coli K-12 has high homology to PD of Brevibacterium lactofermentum. In particular, the 330 serine residue from the N-terminal (³³⁰Ser) of the CM-PD of Escherichia coli K-12 corresponds to the 235 serine residue (²³⁵Ser) of Brevibacterium lactofermentum PD. Sequence Nos. 9 and 10 are synthesized in such a manner that the 330 serine residue becomes a proline residue.

Next, using 1 μ g of the chromosomal DNA and either 300 ng of each of the primers of Sequence Nos. 1 and 10, or 300 ng of each of the primers of Sequence Nos. 8 and 9, PCR was carried out to obtain DNA fragments of 1.3 Kbp and 0.5 Kbp, respectively. The PCR temperature cycle of reaction at 94°C for one minute, at 50°C for 2 minutes and at 72°C for 3 minutes was repeated for 20 cycles using a continuous 5 replication reaction device (Thermal Cycler, manufactured by Perkin Elmer Cetus Co.), according to the method of Erlich et al. [PCR Technology, Stockton Press (1989)]. These DNA fragments were subjected to agarose gel electrophoresis and recovered using a standard DNA recovery kit (Gene Clean, manufactured by Funakoshi Co.).

Separately, using these fragments and the primers of Sequence Nos. 7 and 8, PCR reaction was further 10 carried out to obtain a DNA fragment of 1.8 Kbp. After the 1.8 Kbp fragment was digested with BamHI and PstI, a DNA fragment of 1.7 Kbp was recovered by agarose electrophoresis. Subsequently, the 1.7 Kbp fragment was ligated with the BamHI and PstI digestion product of plasmid pHSG398 (manufactured by Takara Shuzo) using T4 DNA ligase. The ligation product was used to transfet Escherichia coli KA197 strain (pheA). Among the strains resistant to chloramphenicol, the strain in which phenylalanine auxotrophy 15 disappeared was selected, and a plasmid was recovered. The plasmid was named pPHAB. Its nucleotide sequence was determined. This plasmid bears the mutant CM-PD enzyme gene in which the 330 serine residue was substituted with a proline residue.

Also using the same methods mentioned above, 330 serine residue from the N-terminal (β^{30} Ser) of the CM-PD of Escherichia coli K-12 was substituted with an aspartic acid residue. Sequence Nos. 11 and 12 20 were synthesized in such a manner that the 330 serine residue became an aspartic acid residue.

Nos. 11 CCGTCTGGAA GACCGCCCCA T

25 Nos. 12 ATCGGGCGGT CTTCCAGACG G

In this way, the plasmid pPHAD, which bears the mutant CM-PD enzyme gene in which the 330 serine residue was substituted with an aspartic acid, was obtained.

Also using the same methods mentioned above, amino acid residues downstream from β^{30} Ser of the 30 CM-PD of Escherichia coli K-12 were deleted. Sequence Nos. 13 and 14 were synthesized in such a manner that the codon of the 330 serine residue became termination codon.

35 Nos. 13 CCGTCTGGAA TGACGCCCGA T

Nos. 14 ATCGGGCGTC ATTCCAGACG G

In this way, the plasmid pPHATerm, which bears the mutant CM-PD enzyme gene in which the amino acid residues downstream from the 330 serine residue were deleted, was obtained.

40 (3) Construction of a tyrA gene-defected W3110 strain of Escherichia coli K-12

Escherichia coli W3110 strain (acquired from National Institute of Heredity) was spread on a plate medium containing streptomycin to obtain a streptomycin-resistant strain. Next, this strain was cultured in a 45 culture medium in combination with Escherichia coli K-12 ME8424 strain (HfrPO45, thi, relA1, tyrA: Tn10, ung-1, nadB) (acquired from National Institute of Heredity), and the medium was allowed to stand at 37°C for 15 minutes to perform conjugation transfer. Then the medium was applied to a plate medium containing streptomycin, tetracycline and L-tyrosine. The formed colony, i.e., Escherichia coli W3110 (tyrA) strain, was collected.

50 The plasmid pPHAB, pPHAD, and pPHATerm obtained in Example (2)-2 above were used to transform competent cells of the E. coli W3110 (tyrA) strain. The transformant Escherichia coli K-12 [W3110 (tyrA)-/pPHAB] Escherichia coli K-12 [W3110 (tyrA)/pPHAD], and Escherichia coli K-12 [W3110 (tyrA)/pPHATerm] were deposited in the Fermentation Research Institute of the Agency of Industrial Science & Technology of Japan. The deposit numbers are FERM BP-3566, FERM BP-12659, and FERM BP-12662, respectively.

55 (4) Measurement of PD enzyme activity

Escherichia coli K-12 W3110 (tyrA)/(pPHAB) strain was cultured at 37°C for 15 hours in L medium and

the cells were collected by centrifugation. Then, the cells were washed twice with physiological saline, and suspended in 250 mM Tris-hydrochloride buffer (pH 7.5) containing 0.5 mM dithiothreitol under ice cooling. By ultrasonication (20 KHz) for 30 seconds four times, the crude enzyme solution was prepared.

The PD enzyme activity was determined in a conventional manner [cotton, R.G.H. and Gibson, F., Meth. in Enzymol., 17, 564 (1970)]. Using the crude enzyme, the enzymatic reaction was carried out at 37° C for 10 minutes in the presence of 50 mM Tris-hydrochloride buffer (pH 8.2) containing 1 mM barium prephenate and 0.5 mM L-tyrosine. Aqueous sodium hydroxide (1 N) was added to terminate the reaction, and the formed phenylpyruvic acid was measured at an extinction wavelength of 320 nm.

Quantitative determination of protein was made using the Protein Assay Kit (manufactured by Bio Rad Co.), according to the protocol of the manufacturer.

The results presented in Fig. 4 show that the enzyme reaction in strains transformed with the wild CM-PD gene was strongly inhibited in the presence of 0.5 mM L-phenylalanine, whereas strains transformed with a mutant CM-PD gene exhibited almost no inhibition, even in the presence of 5 mM L-phenylalanine.

Further, in the case of the plasmid bearing the wild type enzyme gene, in the absence of L-phenylalanine, the enzyme activity was only 3.5×10^2 U/mg protein. In the case of the mutant CM-PD gene, the activity was 1.5×10^4 U/mg protein. The results show that not only is expression of the mutant type CM-PD enzyme gene released from feedback inhibition by L-phenylalanine in transformants containing the mutant gene, but surprisingly, the amount of enzyme and/or enzyme activity can be increased by roughly two orders of magnitude.

By using the same method above, the PD enzyme activities of Escherichia coli W3110 (tyrA)/(pPHAD) and Escherichia coli W3110 (tyrA)/(pPHATerm) were determined. As the result, the PD enzymes of the both strains were found to be released from feedback inhibition by L-phenylalanine.

Example 3: Production of L-phenylalanine by fermentation

(1) Construction of Escherichia coli K-12 bearing a CM-PD gene alone and in combination with a DS gene, in which the feedback inhibition is released

From pTS-aroG4 bearing the feedback inhibition-released DS gene obtained in Example 1, the aroG4 portion was excised with restriction enzymes EcoRI and HindIII. The fragment was inserted into the cleavage site of pBR322 with EcoRI and HindIII to obtain plasmid pBR-aroG4 (having an ampicillin-resistant marker).

In a separate procedure, pPHAB bearing the feedback inhibition-released CM-PD gene obtained in Example 2 was digested with restriction enzymes BamHI and HindIII to excise the fragment containing the CM-PD gene. This fragment was inserted into the cleavage site of pACYC184 with BamHI and HindIII to construct plasmid pACMAB (selection marker was chloramphenicol resistance). The pACMAB plasmid was used to transform competent cells of Escherichia coli K-12 W3110 (tyrA), yielding transformant W3110 (tyrA)/pACMAB.

Furthermore, the two plasmids pACMAB and pBR-aroG4 were used to transform W3110 (tyrA) yielding transformant W3110 (tyrA)/pBR-aroG4,pACMAB. The transformant W3110 (tyrA)/pBR-aroG4,pACMAB was named AJ 12604 strain and deposited in Fermentation Research Institute of the Agency of Industrial Science & Technology of Japan under the deposit number FERM BP-3579.

(3) Production of L-phenylalanine

The transformant AJ 12604, W3110 (tyrA)/pACMAB, W3110 (tyrA)/pPHAD, W3110 (tyrA)/pPHATerm, and W3110 (tyrA) described above were cultured at 37° C for 24 hours in L-phenylalanine-producing medium (containing 20 g of glucose, 29.4 g of disodium hydrogen phosphate, 6 g of potassium dihydrogen phosphate, 1 g of sodium chloride, 2 g of ammonium chloride, 10 g of sodium citrate, 0.4 g of sodium glutamate, 3 g of magnesium sulfate heptahydrate, 0.23 g of calcium chloride and 2 mg of thiamine hydrochloride in 1 liter of water). The results are shown in Table 2. Quantitative assay was performed by high performance liquid chromatography. An outstanding improvement in the fermentative production of L-phenylalanine using the AJ 12604 strain was obtained.

Table 2

Strain	Amount of L-phenylalanine
W3110(tyrA)	0.1
W3110 (tyrA)/pACMAB	0.5
W3110 (tyrA)/pPHAD	0.5
W3110 (tyrA)/pPHATerm	0.5
AJ 12604	3.8

Example 4: Production of L-tryptophan by fermentation

15 (1) Construction of a plasmid bearing feedback inhibition-released DS

Plasmid pACYC177 (acquired from National Institute of Heredity; ampicillin resistance, 3.6 Kbp) was digested with restriction enzyme XbaI. After the digestion site was made blunt ended by Klenow treatment, an EcoRI linker was ligated therewith using T4 DNA ligase to obtain the plasmid pACYC177E, in which the XbaI site became EcoRI. Next, the plasmid pTS-aroG4 described in Example 1-(2) and 1-(3) above was digested with restriction enzymes EcoRI and HindIII to obtain the fragment containing aroG4. This fragment was ligated with the EcoRI- and HindIII-digested pACYC177E using T4 DNA ligase. Competent cells of the AB3257 strain (described in Example 1) was transformed with the reaction mixture. Among the ampicillin-resistant strains grown, a strain in which auxotrophy for each of L-tyrosine, L-phenylalanine and L-tryptophane disappeared was selected, and a plasmid was extracted. Thus, plasmid pACEG4 (5.1 Kbp) was obtained. An EcoRI-EcoRI fragment containing a gene conferring kanamycin resistance (Kanamycin Gene Block; 1.3 Kbp, manufactured by Pharmacia Fine chemicals) was ligated with the plasmid pACEG4 at the EcoRI site using T4 DNA ligase, thus yielding the plasmid pACKG4 (resistant to ampicillin and kanamycin, 6.4 Kbp). The procedure of the construction of pACKG4 is outlined in Fig. 5.

20 (2) Construction of Escherichia coli K-12 bearing a feedback inhibition-released DS gene and a tryptophan operon

Competent cells of Escherichia coli K-12 AGX6aroP strain (described in U.S. Patent No. 4,371,614, incorporated herein by reference; deposit number: NRRL B-12264) bearing the plasmid pGX50 harboring a tryptophan operon was transformed with the pACKG4 plasmid described above to obtain Escherichia coli AGX6aroP/pGX50,pACKG4. The genotype of Escherichia coli AGX6aroP strain is tna, trpR+, aroP.

25 (3) Production of L-tryptophan

The transformant Escherichia coli AGX6aroP/pGX50,pACKG4 and AGX6aroP/pGX50 described above was cultured at 30°C for 72 hours in L-tryptophan-producing medium (containing 40 g of glucose, 15 g of ammonium sulfate, 1 g of potassium monohydrogen phosphate, 1 g of magnesium sulfate heptahydrate, 0.01 g of ferrous sulfate heptahydrate, 0.01 g of manganese chloride tetrahydrate, 2 g of yeast extract and 40 g of calcium carbonate in 1 liter of water, pH 7). The results are shown in Table 3. Quantitative assay of L-tryptophan was performed by high performance liquid chromatography. An outstanding improvement in the fermentative production of L-tryptophan using the AGXaroP/pGX50,pACKG strain was obtained.

Table 3

Strain	Amount of L-tryptophan Produced (g/l)
AGX6aroP/pGX50	0.15
AGX6aroP/pGX50,pACKG4	0.45

Obviously, numerous modifications and variations of the present invention are possible in light of the

above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

10

(i) APPLICANT: Ajinomoto Co., Ltd.

15

(ii) TITLE OF INVENTION: GENES ENCODING FEEDBACK
INHIBITION-RELEASED ENZYMES, PLASMIDS CONTAINING THE
GENES, AND MICROORGANISMS TRANSFORMED WITH THE PLASMIDS
USEFUL IN PROCESSES FOR PREPARING AROMATIC AMINO ACIDS BY
FERMENTATION

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(iii) NUMBER OF SEQUENCES: 12

25

(2) INFORMATION FOR SEQ ID NO:1:

30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAACCAAGT AAAGCCAAACA

20

15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCCACCTTCAG CAACCAGTTTC

20

35

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

45

(iii) HYPOTHETICAL: NO

50

55

5 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
GTATTTACCC CGTTATTGTC

20

15 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

25 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACTCCGCCGG AAGTGACTAA

20

30 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 948 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

35 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Brevibacterium lactofermentum
(B) STRAIN: AJ 12125

(ix) FEATURE:

40 (A) NAME/KEY: CDS
(B) LOCATION: 1..945

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG	ACC	CAC	CCA	CCA	ATT	GTT	GTG	GCC	TAT	TTG	GGG	CCT	GCC	GGA	ACC	48
Met	Ser	Asp	Ala	Pro	Ile	Val	Val	Ala	Tyr	Leu	Gly	Pro	Ala	Gly	Thr	
5	1				5				10						15	
TTC	ACC	GAA	GAA	GCC	CTC	TAC	AAA	TTT	GCC	GAC	GCC	GGC	GTA	TTC	GGC	96
Phe	Thr	Glu	Glu	Ala	Leu	Tyr	Lys	Phe	Ala	Asp	Ala	Gly	Val	Phe	Gly	
					20				25						30	
10	GAC	GGT	GAG	ATC	GAG	CAG	CTA	CCA	GCC	AAA	TCG	CCA	CAA	GAA	GCT	144
Asp	Gly	Glu	Ile	Glu	Gln	Leu	Pro	Ala	Lys	Ser	Pro	Gln	Glu	Ala	Val	
					35				40						45	
15	GAC	GCG	GTC	CGC	CAC	GGC	ACC	GCC	CAG	TTC	GGC	GTC	GCC	ATC	GAA	192
Asp	Ala	Val	Arg	His	Gly	Thr	Ala	Gln	Phe	Ala	Val	Val	Ala	Ile	Glu	
					50				55						60	
20	AAC	TTC	GTC	GAC	GGC	CCC	GTC	ACC	CCC	ACC	TTC	GAC	GCC	CTT	GAC	240
Asn	Phe	Val	Asp	Gly	Pro	Val	Thr	Pro	Thr	Phe	Asp	Ala	Leu	Asp	Gln	
	65				70				75						80	
25	GGC	TCC	AAC	GTG	CAA	ATC	ATC	GCC	GAA	GAA	GAA	CTC	GAT	ATT	GCC	288
Gly	Ser	Asn	Val	Gln	Ile	Ile	Ala	Glu	Glu	Glu	Glu	Leu	Asp	Ile	Ala	Phe
					85				90						95	
30	TCC	ATC	ATG	GTC	CGG	CCA	GGG	ACT	TCG	CTT	GCC	GAC	GTC	AAA	ACC	336
Ser	Ile	Met	Arg	Pro	Gly	Thr	Ser	Leu	Ala	Asp	Val	Lys	Thr	Leu		
					100				105						110	
35	GCC	ACC	CAC	CCG	GTT	GGG	TAC	CAA	CAA	GTG	AAA	AAC	TGG	ATG	GCA	384
Ala	Thr	His	Pro	Val	Gly	Tyr	Gln	Gln	Val	Lys	Asn	Trp	Met	Ala	Thr	
					115				120						125	
40	ACC	ATT	CCG	GAC	GCC	ATG	TAT	CTT	TCA	GCA	AGC	TCC	AAC	GGC	GCC	432
Thr	Ile	Pro	Asp	Ala	Met	Tyr	Leu	Ser	Ala	Ser	Ser	Asn	Gly	Ala	Gly	
					130				135						140	
45	GCA	CAA	ATG	GTT	GCC	GAA	GGA	ACC	GCC	GAC	GCA	GCC	GCA	GCG	CCC	480
Ala	Gln	Met	Val	Ala	Glu	Gly	Thr	Ala	Asp	Ala	Ala	Ala	Ala	Pro	Ser	
	145				150				155						160	
50	CGC	GCA	GCC	GAA	CTC	TTC	GGG	CTG	GAA	CGC	CTT	GTT	GAT	GAT	GTC	528
Arg	Ala	Ala	Glu	Leu	Phe	Gly	Leu	Glu	Arg	Leu	Val	Asp	Asp	Val	Ala	
					165				170						175	
55	GAC	GTC	CGC	GGC	GCC	CGC	ACC	CGC	TTC	GTT	GCA	GTC	CAA	GCC	CAA	576
Asp	Val	Arg	Gly	Ala	Arg	Thr	Arg	Phe	Val	Ala	Val	Gln	Ala	Gln	Ala	
					180				185						190	
60	GCC	GTT	TCC	GAA	CCG	ACC	GGC	CAC	GAC	CGC	ACC	TCC	GTC	ATT	TTC	624
Ala	Val	Ser	Glu	Pro	Thr	Gly	His	Asp	Arg	Thr	Ser	Val	Ile	Phe	Ser	
					195				200						205	

CTA CCG AAT GTG CCA GGC AGC CTC GTG CGC GCC CTC AAC GAA TTC GCC Leu Pro Asn Val Pro Gly Ser Leu Val Arg Ala Leu Asn Glu Phe Ala 210 215 220	672
5 ATC CGT GGC GTC GAC CTC ACC CGC ATC GAA TCC CGC CCC ACC CGC AAA Ile Arg Gly Val Asp Leu Thr Arg Ile Glu Ser Arg Pro Thr Arg Lys 225 230 235 240	720
10 GTC TTC GGA ACC TAC CGC TTC CAC CTG GAC ATA TCC GGA CAT ATC CGC Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg 245 250 255	768
15 GAC ATC CCC GTC GCC GAA GCC CTC CGC GCA CTC CAC CTC CAA GCC GAA Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu 260 265 270	816
20 GAA CTC GTA TTC GTC GGT TCC TGG CCC TCC AAC CGT GCA GAA GAC AGC Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser 275 280 285	864
25 ACG CCC CAA ACC GAC CAA CTA GCT AAC GTA CAC AAG GCG GAC GAA TGG Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp 290 295 300	912
30 GTT CGC GCA GCA AGC GAA GGA AGG AAA CTT AAC TAG Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn 305 310 315	948

26 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 315 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Asp Ala Pro Ile Val Val Ala Tyr Leu Gly Pro Ala Gly Thr 1 5 10 15
35 Phe Thr Glu Glu Ala Leu Tyr Lys Phe Ala Asp Ala Gly Val Phe Gly 20 25 30
Asp Gly Glu Ile Glu Gln Leu Pro Ala Lys Ser Pro Gln Glu Ala Val 35 40 45
40 Asp Ala Val Arg His Gly Thr Ala Gln Phe Ala Val Val Ala Ile Glu 50 55 60

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Asn Phe Val Asp Gly Pro Val Thr Pro Thr Phe Asp Ala Leu Asp Gln
 65 70 75 80

5 Gly Ser Asn Val Gln Ile Ile Ala Glu Glu Glu Leu Asp Ile Ala Phe
 85 90 95

Ser Ile Met Val Arg Pro Gly Thr Ser Leu Ala Asp Val Lys Thr Leu
 100 105 110

10 Ala Thr His Pro Val Gly Tyr Gln Gln Val Lys Asn Trp Met Ala Thr
 115 120 125

Thr Ile Pro Asp Ala Met Tyr Leu Ser Ala Ser Ser Asn Gly Ala Gly
 130 135 140

15 Ala Gln Met Val Ala Glu Gly Thr Ala Asp Ala Ala Ala Pro Ser
 145 150 155 160

Arg Ala Ala Glu Leu Phe Gly Leu Glu Arg Leu Val Asp Asp Val Ala
 20 165 170 175

Asp Val Arg Gly Ala Arg Thr Arg Phe Val Ala Val Gln Ala Gln Ala
 180 185 190

25 Ala Val Ser Glu Pro Thr Gly His Asp Arg Thr Ser Val Ile Phe Ser
 195 300 305

Leu Pro Asn Val Pro Gly Ser Leu Val Arg Ala Leu Asn Glu Phe Ala
 210 215 220

30 Ile Arg Gly Val Asp Leu Thr Arg Ile Glu Ser Arg Pro Thr Arg Lys
 225 230 235 240

Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg
 245 250 355

35 Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu
 260 265 270

Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser
 275 280 285

40 Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp
 290 295 300

Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn
 305 310 315

50 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 948 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Brevibacterium lactofermentum*
 (B) STRAIN: AJ 12259

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

15	ATGAGCGACGCCAACATTGTTGTCGGCTATTGGGGCTGCCGGAACCTTCACCGAACAA	60
	GCCCTCTACAAATTGCGCACGCCGGCGTATTCGGCGACGGTGAGATCGAGCAGCTACCA	120
	GCCAAATGCCAACAGAACGCTGTCGACGCCGGCCACGGCACCGCCCAGTCGGCGTG	180
20	GTCGCCATCGAAAATTCGTCGACGGCCCCGTACCCCCACCTTCGACGCCCTTGACAC	240
	GGCTCCAACGTGCAAATCATGCCAACAGAACACTCGATATTGCCCTTCCATCATGGTC	300
	CGGCCAGGGACTTCGCTTGCGCACGTCAAAACCCCTGCCACCCACCCGGTTGGTACCAA	360
25	CAAGTGAAAAACTGGATGGCAACCACCAATTCCGGACGCCATCTATCTTCAGCAAGCTCC	420
	AACGGCCGGCGCGCACAAATGGTTGCCAACGGAAACCGCCGACGCCAGCCAGCGCCCTCC	480
	CGCGCAGCCGAACTTCGGACTGGAACGCCCTGTTGATGATGCCCGACGTCCGGC	540
30	GCCCCCACCCGTTCTGTTGACTCCAAGCCAACGCCGTTCCGAAACCGACCGCAC	600
	GACCGCACCTCCGTCAATTCTCCCTACCGAATGTCGCCAGGCACCCCTCGCCGCCCC	660
	AACGAATTGCCATCCGTGCCGTCGACCTCACCCGATCGAACCCGCCCCACCCGCAA	720
35	GTCTTCGGAACCTACCGCTTCCACCTGGACATATCCGGACATATCCGCACATCCCC	780
	GCCGAAGCCCTCCGGCGACTCCACCTCCAAGCGAACACTCGTATTCTCGGGCTTGG	840
	CCCTCCAACCGTGCAGAAGACAGCACGCCAACCGACCAACTAGCTAACGTACACAAG	900
40	GCGGACGAATGGTTGCCAGCAAGCGAACGGAAACTTAACCTAG	948

(2) INFORMATION FOR SEQ ID NO:8:

45 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 315 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

10	Met	Ser	Asp	Ala	Pro	Ile	Val	Val	Ala	Tyr	Leu	Gly	Pro	Ala	Gly	Thr
	1				5				10					15		
15	Phe	Thr	Glu	Glu	Ala	Leu	Tyr	Lys	Phe	Ala	Asp	Ala	Gly	Val	Phe	Gly
		20				25				25				30		
20	Asp	Gly	Glu	Ile	Glu	Gln	Leu	Pro	Ala	Lys	Ser	Pro	Gln	Glu	Ala	Val
	35					40							45			
25	Asp	Ala	Val	Arg	His	Gly	Thr	Ala	Gln	Phe	Ala	Val	Val	Ala	Ile	Glu
	50					55						60				
30	Asn	Phe	Val	Asp	Gly	Pro	Val	Thr	Pro	Thr	Phe	Asp	Ala	Leu	Asp	Gln
	65					70			75				80			
35	Gly	Ser	Asn	Val	Gln	Ile	Ile	Ala	Glu	Glu	Glu	Leu	Asp	Ile	Ala	Phe
		85					90						95			
40	Ser	Ile	Met	Val	Arg	Pro	Gly	Thr	Ser	Leu	Ala	Asp	Val	Lys	Thr	Leu
		100					105						110			
45	Ala	Thr	His	Pro	Val	Gly	Tyr	Gln	Gln	Val	Lys	Asn	Trp	Met	Ala	Thr
		115					120						125			
50	Thr	Ile	Pro	Asp	Ala	Met	Tyr	Leu	Ser	Ala	Ser	Ser	Asn	Gly	Ala	Gly
		130				135						140				
55	Ala	Gln	Met	Val	Ala	Glu	Gly	Thr	Ala	Asp	Ala	Ala	Ala	Pro	Ser	
		145				150				155			160			
60	Arg	Ala	Ala	Glu	Leu	Phe	Gly	Leu	Glu	Arg	Leu	Val	Asp	Asp	Val	Ala
						165			170			175				
65	Asp	Val	Arg	Gly	Ala	Arg	Thr	Arg	Phe	Val	Ala	Val	Gln	Ala	Gln	Ala
					180			185				190				
70	Ala	Val	Ser	Glu	Pro	Thr	Gly	His	Asp	Arg	Thr	Ser	Val	Ile	Phe	Ser
		195				200						205				
75	Leu	Pro	Asn	Val	Pro	Gly	Ser	Leu	Val	Arg	Ala	Leu	Asn	Glu	Phe	Ala
		210				215				220						
80	Ile	Arg	Gly	Val	Asp	Leu	Thr	Arg	Ile	Glu	Pro	Arg	Pro	Thr	Arg	Lys
		225				230				235			240			

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Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg
245 250 255

5 Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu
260 265 270

Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser
275 280 285

10 Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp
290 295 300

Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn
305 310 315

15 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25 (iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
30 (A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 RR1

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCAACAAGCT GGAACGGACG

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35 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

45 (vi) ORIGINAL SOURCE:

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(A) ORGANISM: *Escherichia coli*
(B) STRAIN: K-12 RRI

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCCGATTTA CGGCCCTTGAG

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10 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGTCTGGAA CCACGCCCGA T

21

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
ATCGGGCGTG ATTCCAGACG G

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Claims

1. A microorganism transformed with a recombinant DNA sequence encoding an enzyme which corresponds to prephenate dehydratase in which one or two amino acid residue(s) are substituted with other amino acid residue(s) or one or more amino acid residue(s) are deleted, and wherein feedback inhibition is released.
2. A microorganism as claimed in claim 1, wherein said microorganism is of the genus *Escherichia*, said prephenate dehydratase is chorismate mutase - prephenate dehydratase, derived from the genus *Escherichia* and the serine residue 330 residues from the N-terminus is substituted with another amino acid residue.
3. A microorganism as claimed in claim 2, wherein said serine residue is substituted with a proline residue or an aspartic acid residue.
4. A microorganism as claimed in claim 1, wherein said microorganism is of the genus *Escherichia*, said prephenate dehydratase is chorismate mutase - prephenate dehydratase derived from the genus

Escherichia and the amino acid residues downstream from the 330 serine residue are deleted.

5. A microorganism as claimed in claim 1, wherein said microorganism is a Coryneform bacterium, said prephenate dehydratase is derived from a Coryneform bacterium and the serine residue 235 residues from the N-terminus is substituted.
10. A microorganism belonging to the genus Escherichia transformed with a recombinant DNA sequence encoding an enzyme corresponding to 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase derived from the genus Escherichia, wherein one or two amino acid residue(s) are substituted and feedback inhibition is released.
15. A microorganism as claimed in claim 6, further comprising a recombinant DNA sequence encoding one or more enzymes in the biosynthesis of an aromatic amino acid, wherein feedback inhibition of said enzyme is released.
20. A microorganism as claimed in claim 6 or 7, wherein said 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase is encoded by aroF.
25. A microorganism as claimed in claim 8, wherein the aspartic acid residue 147 residues from the N-terminus or the serine residue 181 residues from the N-terminus of said 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase is substituted with another amino acid residue.
30. A microorganism as claimed in claim 9, wherein said aspartic acid residue is substituted with an asparagine residue.
35. A microorganism as claimed in claim 9, wherein said serine residue is substituted with a phenylalanine residue.
40. A microorganism as claimed in claim 6 or 7, wherein said 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase is encoded by aroG.
45. A microorganism as claimed in claim 12, wherein said one or two amino acid residue(s) of said 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase being substituted are selected from the group consisting of the aspartic acid residue 146 amino acid residues from the N-terminus, the methionine residue 147 amino acid residues from the N-terminus, the proline residue 150 amino acid residues from the N-terminus, the alanine residue 202 amino acid residues from the N-terminus, and both the methionine residue 157 amino acid residues from the N-terminus and the alanine residue 219 amino acid residues from the N-terminus.
50. 14. A microorganism as claimed in claim 13, wherein said aspartic acid residue 146 amino acid residues from the N-terminus is substituted with an asparagine residue, said methionine residue 147 amino acid residues from the N-terminus is substituted with an isoleucine residue, said proline residue 150 amino acid residues from the N-terminus is substituted with a leucine residue, said alanine residue 202 amino acid residues from the N-terminus is substituted with a threonine residue, said methionine residue 157 amino acid residues from the N-terminus is substituted with an isoleucine residue and said alanine residue 219 amino acid residues from the N-terminus is substituted with an threonine residue.
55. 15. A microorganism as claimed in claim 7, wherein said enzyme is chorismate mutase-prephenate dehydratase.
16. A microorganism as claimed in claim 7, wherein said enzyme in the biosynthesis of an aromatic amino acid is a tryptophan operon.
17. A process for preparing an aromatic amino acid which comprises culturing a microorganism claimed in claims 1, 2, 4, 5, 6 or 7 in a medium, and isolating the aromatic amino acid produced.
18. A process for preparing an aromatic amino acid as claimed in claim 17, wherein said aromatic amino acid is L-phenylalanine or L-tryptophan.

19. A recombinant DNA sequence encoding an enzyme which corresponds to prephenate dehydratase derived from a Coryneform bacterium, 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase, or chorismate mutase-prephenate dehydratase derived from the genus Escherichia, in which one or two amino acid residue(s) are substituted with other amino acid residue(s) or one or more amino acid residue(s) are deleted, and wherein feedback inhibition is released.

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20. An enzyme which corresponds to prephenate dehydratase derived from a Coryneform bacterium, 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase, or chorismate mutase-prephenate dehydratase derived from the genus Escherichia, wherein one or two amino acid residue(s) are substituted or one or 10 more amino acid residue(s) are deleted, and feedback inhibition is released.

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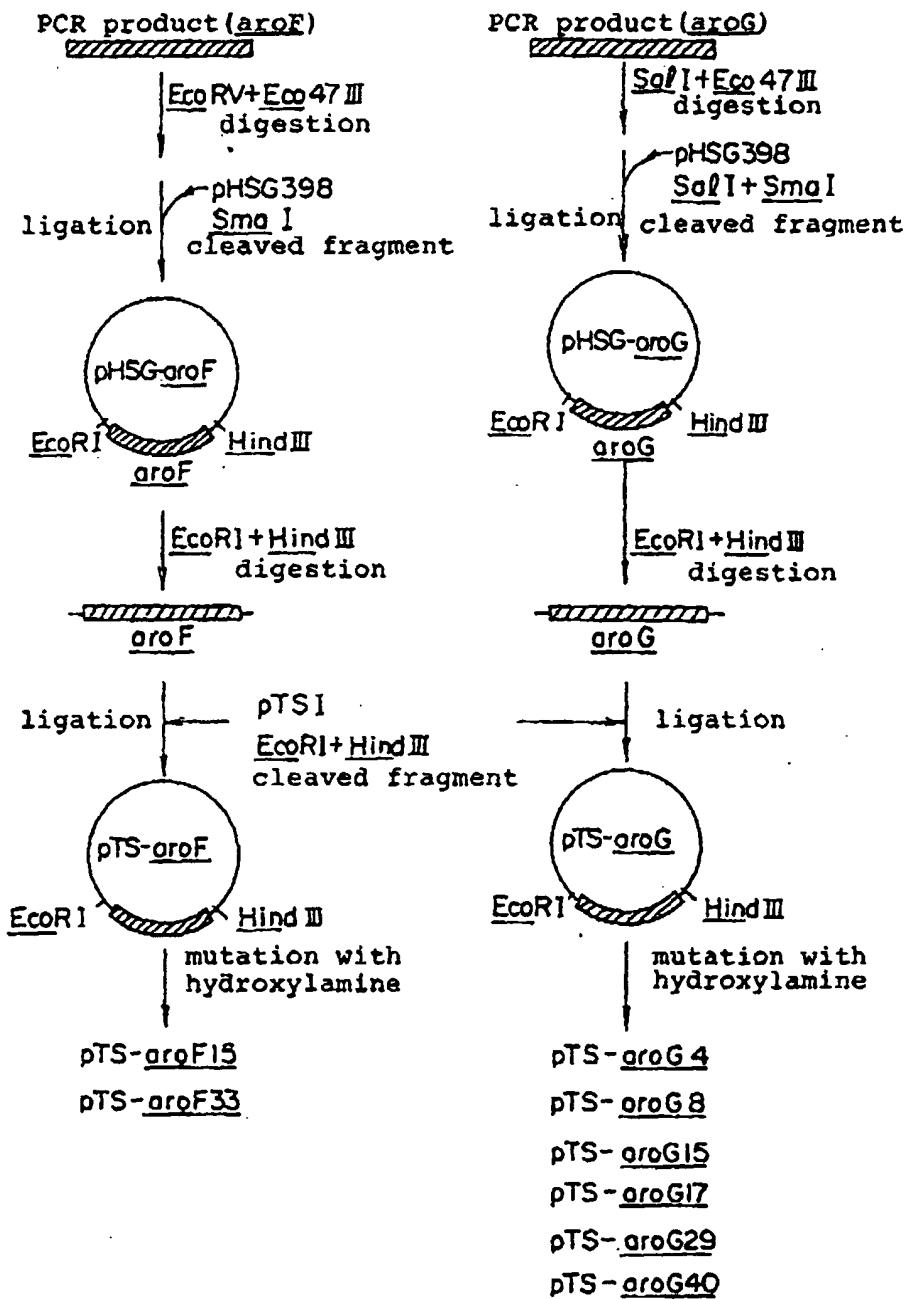
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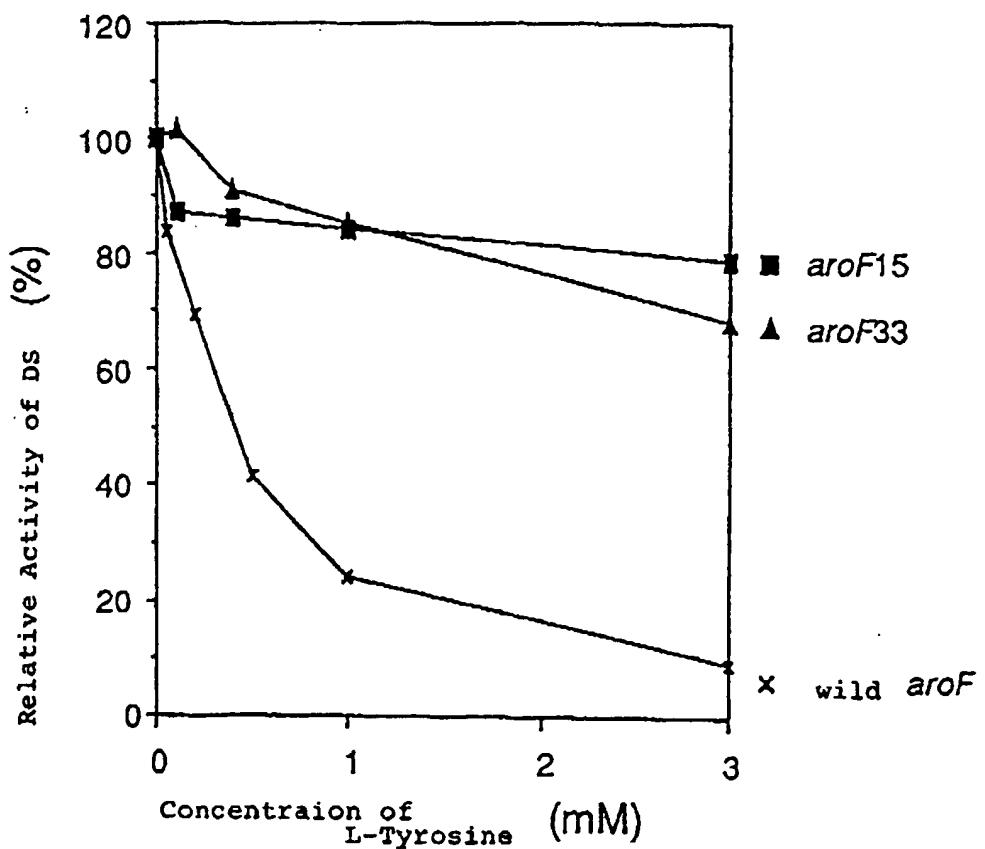
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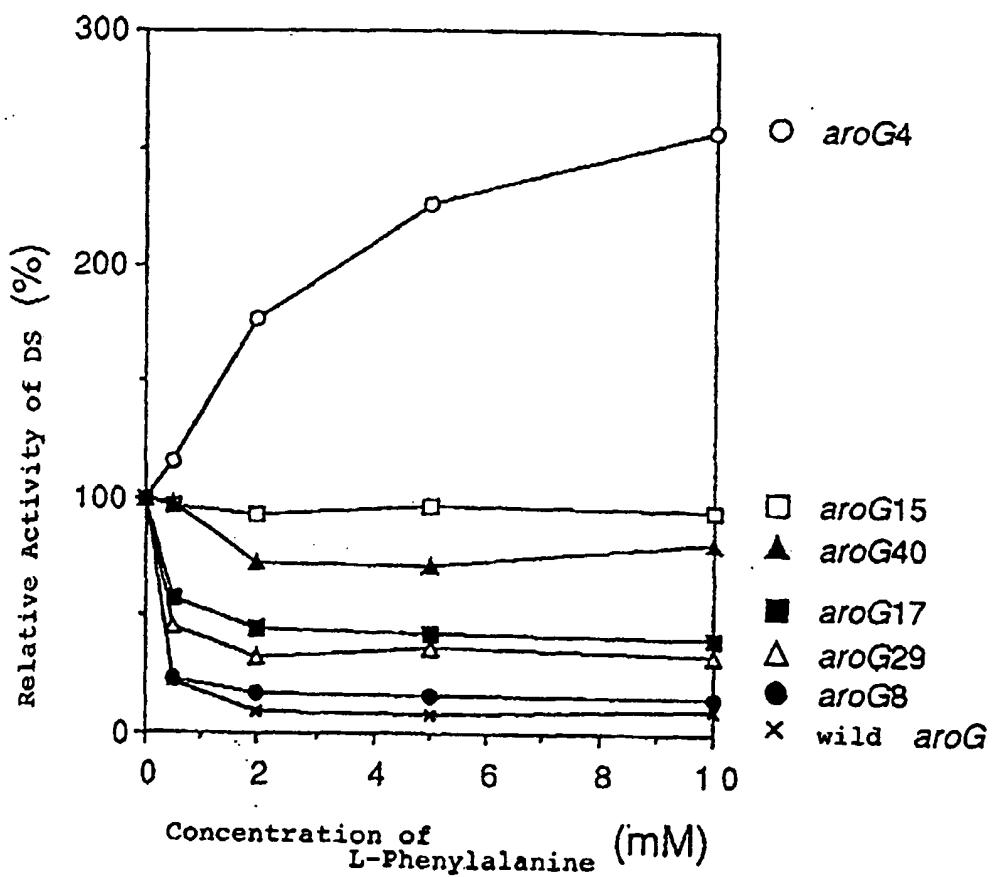
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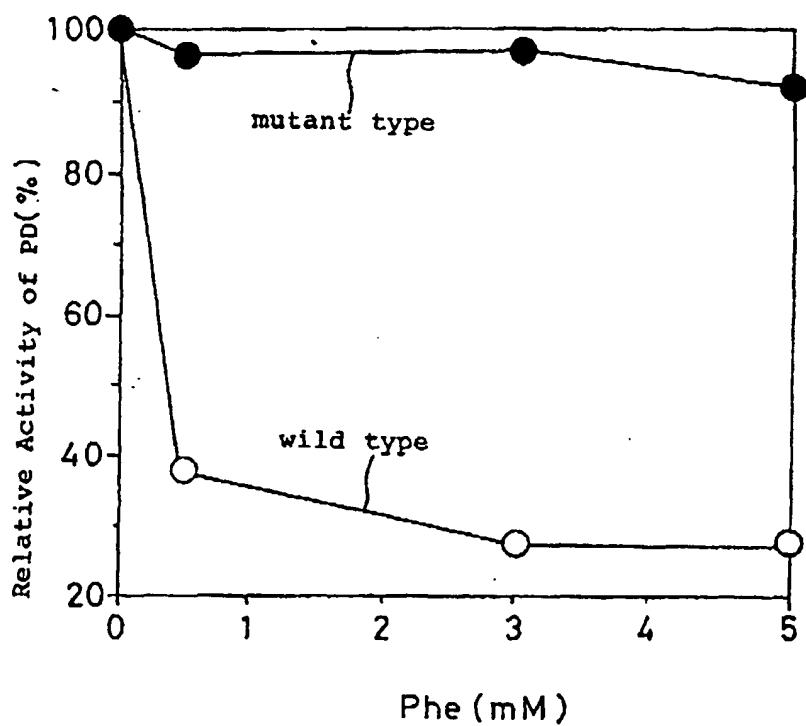
(FIG. 2)



(FIG. 3)



(FIG. 4)



(FIG. 5)

